Spotlight

Food & Beverage Analysis

Mycotoxins are a group of toxic metabolites produced naturally by certain species of fungi. They contaminate food and feed products and pose a potential threat to human and animal health through ingestion. Most Asian and Australasian countries are located in the tropics and subtropics, where there is increased growth of fungi due to the high temperatures and humidity. As a result, these areas face serious mycotoxin contamination issues. In response, regulatory bodies in the region have introduced strict legislation to set mycotoxin limits for foods and feed. However, most mycotoxins are toxic in very low concentrations, requiring sensitive and reliable methods for their detection in order to ensure regulatory compliance.

This article provides a brief overview of current legislation enforced in Asia and Australasia and presents benchtop high-resolution mass spectrometry (MS) as the simplest and most powerful available method for monitoring the presence of mycotoxins in food and feed. Recently collected data demonstrates the benefits of the technology for reliable mycotoxin screening analyses.

"Positive and negative acquisition can be performed in a single run while also allowing for post acquisition data mining."

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Examining the Benefits of High Resolution Mass Spectrometry for High Throughput Screening of Priority Mycotoxins in Food Samples

INTRODUCTION

Mycotoxin-producing organisms can infect and colonise various agricultural crops in the field and during storage. Environmental factors such as temperature and humidity influence the occurrence of these toxins on grains, nuts and other commodities susceptible to mould infestation. In addition, any crop that is stored for more than a few days is a target for mould growth and therefore mycotoxin formation. Mycotoxins present a health risk because most mycotoxins are relatively stable compounds that are not destroyed by food processing such as cooking or freezing. Although the generating organisms might not survive processing, the toxin can still be present.

Recently, mycotoxins have been identified by the World Health Organisation (WHO) as significant sources of food-borne illnesses. They can cause acute health conditions, such as immediate toxic response, immunosupression, necrosis of liver cells, sickness, vomiting and abdominal pain, as well as having chronic teratogenic, estrogenic, hepatotoxic, nephrotoxic and carcinogenic effects. Some of the most dangerous mycotoxin groups are aflatoxins found in cereals and nuts, ochratoxins found in cereals, patulin found in fruits and vegetable and fusarium toxins found in corn and wheat.

A recently published survey [1] about the occurrence of mycotoxins in Asia, conducted by Biomin GmbH together with Romer laboratories in Singapore, reported that 58% of 960 raw feed samples were contaminated with a type of mycotoxins known as fumonisins, produced by several species of fusarium moulds. Reports such as this highlight the need to safeguard human and animal health from mycotoxin contamination. In order to ensure that mycotoxins do not contaminate the food supply, legislation has been introduced across a number of countries worldwide, including in Asia and Australasia.

LEGISLATIVE FRAMEWORK

An international inquiry [2] on mycotoxins, initiated by the Dutch National Institute for Public Health and the Environment in 2002, revealed that a total of 26 countries in Asia and Australasia enforce specific mycotoxin regulations.

Legislation for total aflatoxin compounds is more prevalent for food, whereas regulations for the specific aflatoxin B1 dominate for feed analysis. According to the findings of the inquiry, Australia and New Zealand have harmonised mycotoxin regulations with common limits being applied for total aflatoxins in foods such as peanuts and tree nuts. In addition, the harmonised regulations include limits for another type of mycotoxin, phomopsin, in products derived from lupin seed products as well as for food products containing agaric acid such as those derived from mushrooms and alcoholic beverages.

This inquiry further revealed that China and the Islamic Republic of Iran have the most extensive and detailed regulations for acceptable mycotoxin levels. In addition, most of the member countries of the Association of Southeast Asian Nations (ASEAN) have now set specific regulations for mycotoxins. While harmonised regulations are not yet established by ASEAN, an ASEAN Task Force on Codex Alimentarius has taken a common position to support a unified level of 0.5mg/kg for aflatoxin M1 in milk.

In order to comply with the different regulations for acceptable levels of various types of mycotoxins, it is essential that the food and feed safety industries in Asia and Australasia have access to an analytical method that can efficiently screen and accurately reveal adulteration of products. The chosen analytical technique will play a key role in the typical mycotoxin method workflow.

TYPICAL MYCOTOXIN METHOD WORKFLOW

The typical method that most laboratories use to perform mycotoxin screening analyses consists of four main steps. The first step is the extraction of mycotoxins from a food sample using different approaches. The most common approach involves using a mixture of acetonitrile and water, with a ratio of 80-20%. Following extraction and prior to liquid chromatography/mass spectrometry (LC/MS) analysis, it is typically necessary to carry out a clean up of the sample, which involves the separation of mycotoxins from the matrix that could later interfere with the final determination. This is usually done using immunoaffinity clean-up columns. These columns specifically attract mycotoxins while all the other compounds are flushed away. Mycotoxins that are retained in the columns are eluted after the matrix has been removed.

For HPLC or UHPLC analysis of mycotoxins with conventional detectors like UV or FL typically a derivatisation step has to be performed. This is of particular importance for some mycotoxins, such as fumonisins, trichothecenes and aflatoxins B1 and G1. The final step is detection. This can be done using a variety of methods, however since most mycotoxins are toxic in very low concentrations, a sensitive and reliable detection method is required.

TRADITIONAL ANALYTICAL APPROACHES

A wide range of analytical tools exist for the food and feed safety industry, but the real challenge is finding a technique that can efficiently screen and accurately reveal adulteration. Liquid chromatography (LC) with fluorescent detection (FLD) has been efficiently used for more than two decades, and in the last five years LC has been increasingly coupled with MS/MS. Quick screening methods based on immunoassay analysis, such as ELISA, are also very popular. Immunoassays are simple and rapid, but they lack sensitivity compared to chromatographic methods and may lead to false negatives. In addition, cross-reactivity of compounds can result in false positives.

The last few years have seen a growing trend towards full scan MS experiments in screening analysis compared to the target LC/MS/MS analysis. Such approaches are performed using high performance time-of-flight (TOF) instruments, with typical mass accuracies of 5 ppm and resolving power of a maximum of 15,000 FWHM, coupled to U-HPLC. However, in complex matrices such as food and feed, this rather limited mass resolving power leads to the risk of inaccurate mass measurements caused by unresolved background matrix interferences [1, 2].

In response, latest technological advancements have focused on the development of an innovative high resolution MS technique using a benchtop LC/MS system capable of achieving routine ultra-high mass accuracy below 2 ppm and mass resolving power of up to 100,000 FWHM.

HIGH RESOLUTION MS

The main advantage of this sophisticated MS technique is its very simple setup and tuning with all data being acquired only in full scan mode, without the need for specific parameters for each compound under investigation. This allows users to conduct an additional search for further possible contaminants after the sample run has been completed. The high resolving power of the system offers greatly enhanced detection capabilities, improving analytical accuracy and providing a high degree of confidence in the results. In addition, high resolution of 100,000 FWHM improves the chromatographic peak shape and facilitates its quantitation.

High resolution MS a simple sample preparation process which consists of the extraction of mycotoxins from raw materials without any specific clean up. As such, the method is capable of analysing a broad range of mycotoxins in a variety of matrices. Offering unique quantitation and target screening capabilities, ultra-high resolution MS enables analysts to monitor target compounds. Non-target screening is also possible eliminating the need to focus only on particular compounds and instead allowing scientists to search for unknowns in the material.

Positive and negative acquisition can be performed in a single run while also allowing for post acquisition data mining. Excellent stability and robustness are additional benefits of the method. Ultra-high resolution MS uses a narrow mass extraction window to improve the selectivity of chromatographic detection. The narrower the window is, the better the selectivity achieved, resulting in elimination of chemical background and chemical noise. A further significant advantage of the technique is its ability to achieve very good repeatability of injections. Repeatability is very important since, according to the European Commission Decision of C1 14 August 2002 [1], quantitation methods must offer repeatability below 20%.

Compared to TOF MS solutions, high-resolution MS systems provide lower detection limits and improved sensitivity. Additionally, the high resolving power of the systems minimises interferences. This cannot be achieved with the low-resolution capability offered by TOF MS instruments.

An experiment was developed to demonstrate the superiority of the method for monitoring the presence of priority fusarium mycotoxins as well as their masked forms in samples collected during beer production.

EXPERIMENTAL

A benchtop LC/MS system (Exactive™, Thermo Fisher Scientific, Bremen, Germany) was coupled to a U-HPLC chromatograph (Accela™, Thermo Fisher Scientific, San Jose, USA) to evaluate a mixture of mycotoxins, including masked mycotoxins in cereal extracts.

A 9 min gradient was applied to a 100mm x 2.1mm C18 column (2.1mm x 100mm x 1.8µm) with 5mM ammonium formate/methanol eluents. Mass measurements were performed at different mass resolving power settings ranging from 25,000 up to 100,000 FWHM.

NON TARGET SCREENING OF BEER USING SIMPLIFIED SAMPLE PREPARATION

The variability of analytes potentially present in the samples requires the simplification of the sample preparation step to avoid losses of analytes during sample extraction and clean up. In order to avoid missing mycotoxins and their metabolites, any sample adjustment (e.g. immuno-affinity or solid phase extraction clean-up) could not be used. The capabilities of single stage, high resolution MS for a non-targeted screening approach were tested on beer samples spiked with known amounts of mycotoxins and extracted using a simple sample preparation procedure based on the precipitation of beer with acetonitrile and centrifugation.

The Exactive MS system, using high mass resolving power of 100,000 FWHM and a narrow extraction window of 3 ppm, was employed for the analysis of prepared samples. *Figure 1* presents example of extracted ion chromatograms of deoxynivalenol at concentration level of 100µg/L. The results demonstrate the high selectivity and sensitivity of the technique compared to TOF mass spectrometry. In such a complex matrix, it would have been impossible to detect DON at low levels using a TOF instrument with resolving power below 12,000 FWHM.

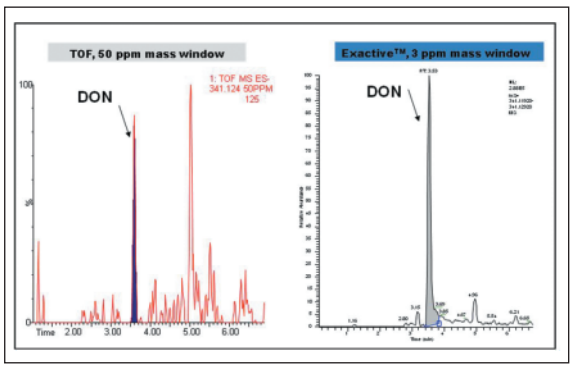

Figure 1: The comparison of the detection capability of TOF technology running with resolving power 12,000 FWHM (left) and Exactive system running at 100,000 FHWM resolving power (right).

Similarly, significant improvement of the identification and quantitation of HT-2 toxin in a beer sample was observed when a high mass resolving power of 100,000 FWHM was employed (*Figure 2*). The peak shape of HT-2 toxin has significantly improved using ultra-high resolving power (A). This was due to better separation of the mass peaks of HT-2 toxin and interfering species. Also, the high resolving power and excellent mass accuracy improved the selectivity of the system by using very narrow mass extraction windows down to 2 ppm (B)

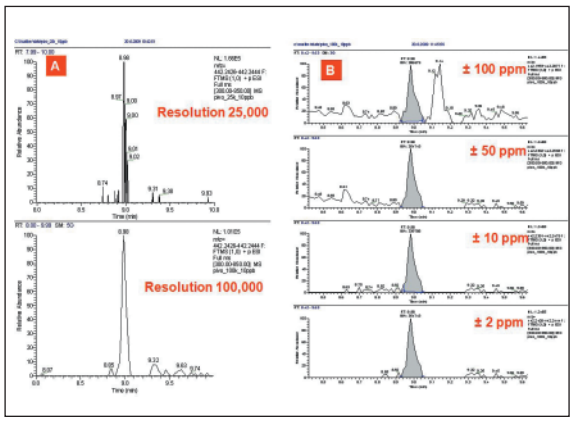

Figure 2: The effect of mass resolution (A) and extraction window width (B) on the quality of chromatographic data and system selectivity. XICs of HT-2 toxin at concentration level 10ng/mL in beer matrix.

For routine use of the system in the mycotoxin analysis field, a wide linear dynamic range and the linearity of calibration for the analysed compounds should be maintained. During the experiments it was found that the system can maintain very good linearity of a several orders of magnitude. The calibration curve of zearalenone shown in *Figure 3* demonstrates the excellent linearity and dynamic range achieved during analysis of a series of calibration standards.

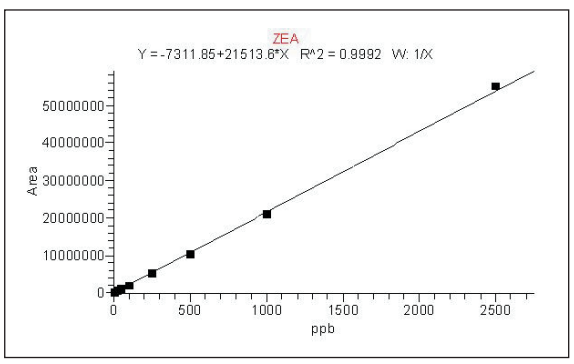

Figure 3: Linearity of the calibration of zearalenone in the calibration range of 1-2500ng/mL.

CONCLUSION

The thorough screening of mycotoxins is required in the regulated environments of food and feed analysis since the presence of these toxins can result in serious adverse effects for humans and livestock. Single stage, full scan MS using Exactive demonstrates ultra high mass resolving power combined with good mass accuracy and selectivity for routine screening and quantitation of mycotoxins in food and feed samples.

Because of the mass selectivity, even in very complex matrices, the superior method results in a simplified sample preparation procedure and increases the number of detected compounds over a wide range of concentrations. In addition, ultra-high resolution MS surpasses conventional TOF MS technology, providing significantly lower detection limits and greatly improved sensitivity while also eliminating background matrix interferences. Further studies and research on this topic are being carried out and the results of the work will be published in a peer reviewed journal in the near future.

Acknowledgements

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[1] Rodriguez, I. and Wegleitner, K. (2008) Biomin Mycotoxin Survey 2007. Asian Pork Magazine June/July 2008.

[2] FAO Corporate Document Repository, Worldwide regulations for mycotoxins in food and feed in 2003, http://www.fao.org/docrep/007/y5499e/y5499e07.htm

[3] Van der Heeft et al., J Am Soc Mass Spectrom 20 (2009), 451–463

[4] Kellman M. et al, in press

[5] Commission Decision of C1 14 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results; http://www.bsmi.gov.tw/wSite/public/Attachment/f1224039659469.pdf